



STUDY OF CHLAMYDIA TRACHOMATIS AND ITS ASSOCIATION WITH OTHER VAGINAL FLORA PATTERNS IN WOMEN ATTENDING A TERTIARY CARE HOSPITAL IN NAVI MUMBAI

Mrinangka Deb¹ | Dr. A. D. Urhekar² | Dr. Jayanti Mania³ | Shilpi C. Kerkar⁴

¹ PhD Scholar, Microbiology Department, MGM Medical College & Hospital, Kamothe, Navi Mumbai, 410209.

² HOD, Microbiology Department, MGM Medical College & Hospital, Kamothe, Navi Mumbai, 410209.

³ Pramanik, Scientist F / Deputy Director (Sr Grade), Department of Infectious Disease & Biology, National Institute of Research in Reproductive Health. Parel, Mumbai, 400030.

⁴ Technician C, Department of Infectious Disease & Biology, National Institute of Research in Reproductive Health. Parel, Mumbai, 400030.

ABSTRACT

Background

Screening women for Chlamydia trachomatis infection in developing countries is highly desirable because of asymptomatic infection and consequent sequel resulting from such infection. Diagnosis of this bacterium is only limited to certain research centers and is not widespread. Moreover little is known about the association of C. trachomatis with other Sexually Transmitted Agents.

METHODS

In this cross-sectional pilot study, 55 women attending the Gynecology Out Patient were screened for the detection of C. trachomatis infection. Polymerase chain reaction was used to diagnose current C. trachomatis infection and ELISA for past infections. In addition patients were also screened for Bacterial vaginosis, Candida, N. gonorrhoea, Syphilis and Vaginal Trichomoniasis to try and establish a casual relationship between C. trachomatis and other reproductive tract agents.

RESULTS

Fifty five women were screened for C. trachomatis as well as other Reproductive Tract Agents of which only 5 patients showed evidence of past C. trachomatis infection, acute infection was not observed. 3 were positive for Bacterial Vaginosis (BV), 8 had Vaginal Candidiasis while there was only 1 case each positive for Syphilis and Vaginal Trichomoniasis. Coinfection of C. trachomatis with BV was observed in only one case. N. gonorrhoea was not detected in any of the samples.

CONCLUSION

The absence of acute C. trachomatis as well as N. gonorrhoea infection in our study could only be attributable to over the counter prescription drugs/antibiotics that are easily available and may have been taken by the patients before coming to the hospital. Self medication not only results in resistance but also promotes persistence of such pathogens.

KEYWORDS: C. trachomatis, Sexually Transmitted Infections, Rapid Plasma Reagin tests, ELISA.

INTRODUCTION

Chlamydia trachomatis is an obligate intracellular human pathogen. It is one of four bacterial species in the genus *Chlamydia*. Other species of *Chlamydia* are *C. psittaci*, *C. pneumonia* and *C. picorum*.^[1] *Chlamydia* causes two types of genital infections: Different urogenital syndromes caused by the oculogenital serotypes D to K, collectively referred to as "genital chlamydiasis" and LGV caused by serotypes L1, L2 and L3.^[2] *Chlamydia* is a major infectious cause of human genital and eye disease. It is estimated that about 1 million individuals in the United States are infected with chlamydia.^[3] Those who have an asymptomatic chlamydia infection that is not detected by their doctor, approximately half will develop pelvic inflammatory disease (PID) in females.

Though most women with *Chlamydia* infection are asymptomatic or have minimal symptoms, some develop salpingitis, endometritis, pelvic inflammatory disease (PID), ectopic pregnancy, abortions and tubal factor infertility. It is associated with an increased risk for the transmission or acquisition of HIV as well as other STIs (Sexually Transmitted Infections) and is also attributed to be a risk factor for the development of cervical carcinoma.^[4]

According to the World Health Organization (WHO), 101 million *chlamydial* infections are detected annually worldwide.^[5] Recent studies from India have revealed the prevalence of *C. trachomatis* infection to be 23% in gynaecology outpatient department (OPD) and 19.9 per cent in STD patients.^[6,7] It has been recovered from 30-60 per cent cases of salpingitis and PID patients in India, while sero-prevalence is shown to be higher in at least one recent study.^[8,9] An estimated 15-40 per cent of women with cervical *chlamydial* infections develop PID.^[10] 20% of women who develop PID become infertile, 18% develop chronic pelvic pain and 9% have a tubal pregnancy.^[11]

Chlamydia trachomatis infection and its association with other vaginal infections namely Bacterial Vaginosis, Gonorrhoea, Syphilis, Trichomoniasis as well as Vaginal Candidiasis has not been studied in our hospital.

This pilot study was conducted to determine the incidence of *C. trachomatis* and its correlation with other sexually transmitted disease (STD) causing organisms

in a tertiary care hospital in Navi Mumbai.

MATERIAL AND METHODS

In this pilot cross-sectional study carried out between June 2015 to August 2016, 55 samples were obtained from women attending the Gynecology Out Patient Department (OPD) of MGM Medical College and Hospital, Kalambohi, Navi Mumbai. The group comprised of women with histories of recurrent spontaneous abortion (RSA, n=4), pre term delivery (n=3), infertility (n=5), signs and symptoms of lower genital tract infections (LGTI, n=37), pregnant women (n=6) attending the antenatal care (ANC) unit. Ethics Committees of the institute approved the study. Each woman was informed about the study and a written consent was obtained from all the women before enrollment.

For this pilot study two vaginal swabs were drawn from each patient for Trichomonas, Candida and bacterial vaginosis (BV) testing. Two more swabs were drawn from the cervix for *Neisseria gonorrhoeae* and Chlamydia testing. In addition a blood sample was obtained to perform serological test for chlamydia. Wet mounts of Vaginal Swabs was used for the detection of *T. vaginalis*. For detection of BV infection, specimens of vaginal exudates were collected with a swab from the posterior fornix of the vagina. Gram stain was performed. A standardized 0-10 scoring system (Nugent's criteria) was used to evaluate BV on the basis of the presence of large Gram-positive rods (*Lactobacilli*), small Gram-negative rods (*Gardnerella*) and *Mobiluncus*. A Nugent's score of ≥ 7 was considered as positive for BV. Smears were also examined for Neutrophils.

For detection of Vaginal Candidiasis and *N. gonorrhoea* endocervical swabs as well as Vaginal Swabs were obtained and Gram stain was performed. The presence of Gram Positive Budding Yeast cells with pseudohyphae was considered to be positive for Vaginal Candidiasis while the presence of Gram Negative Intracellular Diplococci was considered to be positive for *N. gonorrhoea*.

In addition cultures were also put up in Modified Thayer Martin and SDA (Saborauds Dextrose Agar) for the isolation of *N. gonorrhoea* and Candida species respectively. The isolates were identified on the basis of Biochemical Reactions and Germ Tube Test.

All serum samples were subjected to RPR (Rapid Plasma Reagin tests), Tulip Diagnostics, India tests for detection of *Treponema pallidum* as well anti-*chlamydial* IgG antibody detection by ELISA (Novatech Immunodiagnostica, Germany) for detection of past *C.trachomatis* infection. In order to rule out active *C.trachomatis* infection conventional PCR was carried out on the DNA extracted from the cervical specimens. All samples were first subjected to both Quantitative and Qualitative DNA analysis and Beta Globin Gene PCR was also run on all the samples to act as an inbuilt control.

RESULTS and DISCUSSION

Fifty five women were tested for past as well as present/acute *C.trachomatis* (CT) infection as well as other reproductive tract infections. The participants were between 21 to 39 years old with a median age of 29 years. The number of women in the different clinical groups, their age and the infection rate in each group is presented in table 1.

TABLE 1
Incidence of vaginal infections in various clinical groups.

Clinical Groups	Participant (n=55)		Age in Years		CT PCR	CT IgG	Other Infections				BV
	N	n%	Range	Median			Candida sps	T.vaginalis	N.gonorrhoea	Syphilis	
Infertility	5	9	21-33	27	0	1	4	0	0	0	1
LGTI	37	67	25-39	32	0	3	1	1	0	0	2
Abortions	4	7	28-33	30.5	0	1	0	0	0	1	0
ANC	6	11	29-30	29.5	0	0	2	0	0	0	0
Pre Term Delivery	3	5.4	29	29	0	0	1	0	0	0	0

Total Number of Samples = 55

CT IgG - *Chlamydia trachomatis* IgG (ELISA)

ANC - Antenatal Care

LGTI - Lower genital tract infection.

In our pilot study a total of 55 samples (5 Infertility cases, 37 Lower Genitourinary Tract Infection Cases, 4 Abortions, 3 Pre Term Delivery and 6 ANC cases) were examined for *C.trachomatis* infection as well as other reproductive tract infections. Of these 55 samples 5 cases were positive for *C.trachomatis* IgG antibodies denoting past infection, 3 cases were positive for BV (Bacterial Vaginosis), 8 cases were positive for Vaginal Candidiasis and 1 case each positive for Syphilis and Trichomoniasis. No cases of Gonorrhea were detected. All samples were first subjected to Beta Globin PCR to act as PCR Control. CT-PCR was then carried out on all the samples. All samples were negative for CT-PCR. Indicating absence of recent/acute infection by the bacteria. *C.trachomatis* was most commonly associated with the LGTI Group. Coinfection was observed in only 1 case where the patient presenting with Primary Infertility was positive for both BV and CT-IgG.

Sexually transmitted infections (STIs) are a public health problem globally. About 450 million new infections of the four main curable STIs are reported annually. These include Chlamydia, N.gonorrhoea, Syphilis and Trichomoniasis^[12]. Transmission occurs sexually, but can also occur vertically, during pregnancy from mother to child and through blood products or tissue transfer. There are over 30 pathogens that can be transmitted sexually. These include bacteria, viruses, and parasites.

Sexually Transmitted Infections can be broadly classified into curable and non-curable infections. Among the non-curable are the viral infections caused by Human Immunodeficiency Virus (HIV), Herpes Simplex Virus types 1 and 2 (HSV-1 and HSV-2) and Human Papilloma Virus. Some of the causes of the curable STIs include *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoea*, *Treponema pallidum*, *Haemophilus ducreyi* and *Lymphogranuloma venereum*^[13]. Many other organisms that are not deemed sexually transmitted have also been documented to be at least occasionally transmitted through that route, such as *Neisseria meningitidis*, *Haemophilus parainfluenzae*, *Bacterial Vaginosis agents* and Epstein-Barr virus. Sexually transmitted infections can present as asymptomatic infection or as overt disease which may manifest as genital ulceration, discharge or inguinal swelling. Both symptomatic and asymptomatic disease can lead to chronic infections and delayed consequences such as infertility, ectopic pregnancy, cancer of the cervix, and untimely death of infants and adults. Despite the risk posed by these infections, STIs are poorly addressed in many settings, especially in developing countries where the need is greater.

IMAGES

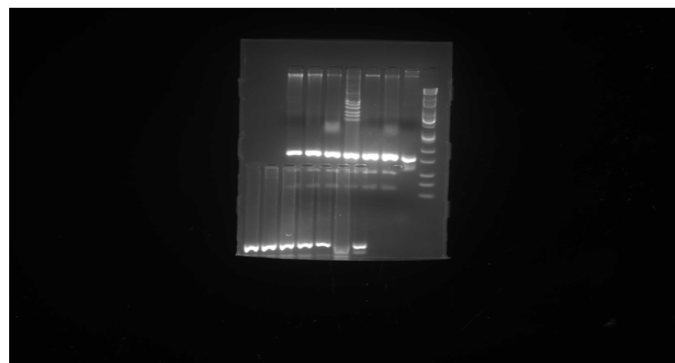


FIGURE 1- C.trachomatis PCR

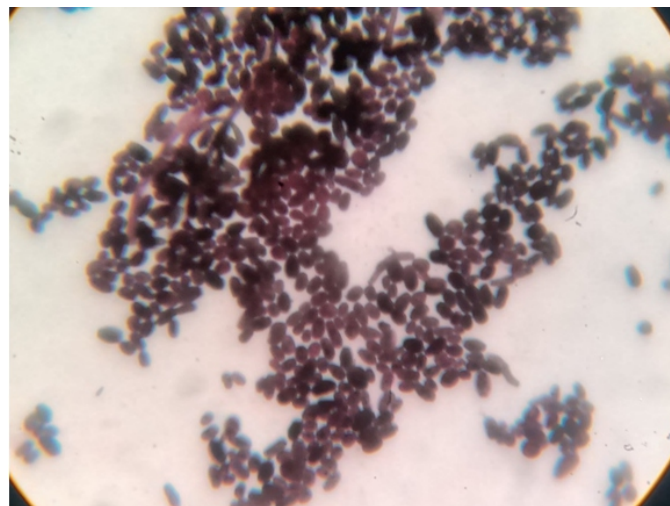


Figure 2-Gram Stain Showing Budding Yeast Cells with Pseudohyphae.

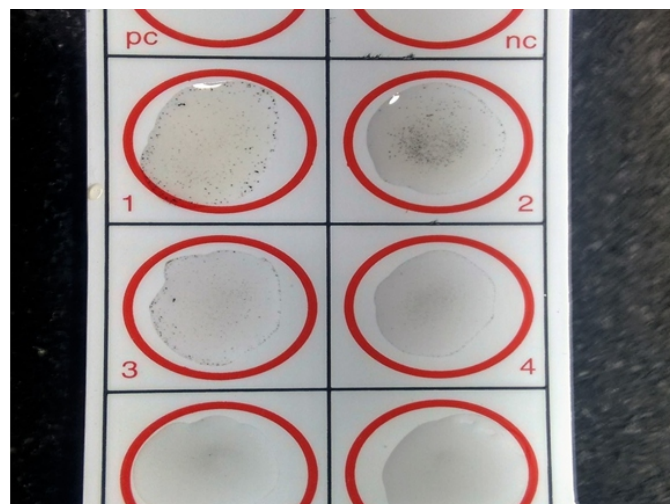


Figure 3-Positive (Rapid Plasma Reagin)RPR Test.

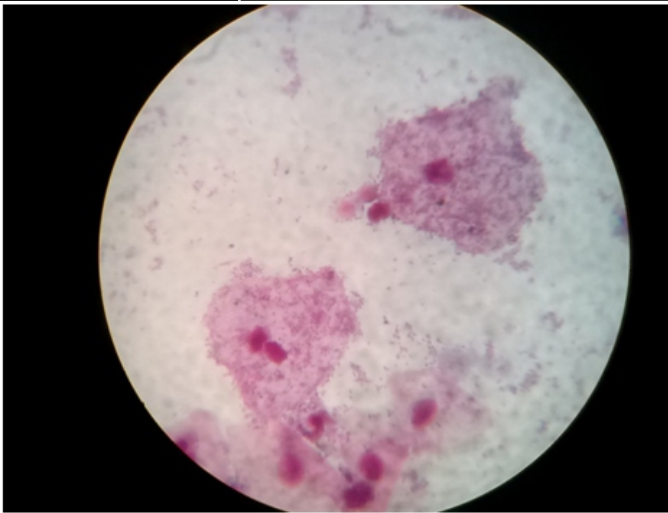


Figure 4- Gram Stain Showing Vaginal Clue Cells.

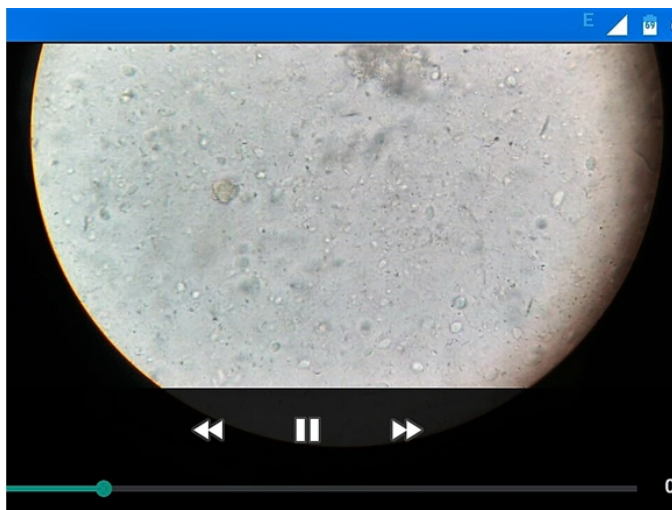


Figure 5-Wet mount showing Vaginal Trichomoniasis

REFERENCES

1. Ryan KJ, Ray CG (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 463–70
2. Ananthanarayan & Paniker's – Text book of Microbiology 8th Edition, Page 419.
3. Chlamydia fact sheet from the Centers for Disease Control and Prevention
4. Wagenlehner FM, Naber KG, Weidner W (2006). "Chlamydial infections and prostatitis in men". BJU Int. 97 (4): 687–90
5. Global prevalence and incidence of selected curable sexually transmitted diseases: Overview and estimates. Geneva: World Health Organization; 2011.
6. Patel LA, Sachdev D, Nagpal P, Chaudary U, Sonkar AS, Mendiratta LS, et al. Prevalence of Chlamydial infection among women visiting a gynaecology outpatient department: evaluation of an in-house PCR assay for detection of Chlamydia trachomatis. Ann Clin Microbiol Antimicrob 2010; 9 : 24-33.
7. Malhotra M, Bala M, Muralidhar S, Khunger N, Puri P. Prevalence of sexually transmitted infections in patients attending a tertiary care hospital in North India –a retrospective Study. Indian J Sex Transm Dis 2008; 29 : 82-5.
8. Mittal A. Genital chlamydiasis. ICMR Bull 1992; 22 : 103-6.
9. Vidhani S, Mehta S, Bhalla P, Bhalla R, Sharma VK, Batra S. Seroprevalence of Chlamydia trachomatis infection amongst patients with pelvic inflammatory diseases and infertility. J Commun Dis 2005; 37 : 233-8.
10. Hills S, Black CM, Newhall J, Walsh C, Groseclose SL. New opportunities for chlamydia prevention: applications of Science to public health Practice. Sex Transm Dis 1995; 22 : 197-202.
11. Miller EK. Diagnosis and treatment of Chlamydia trachomatis infection. Am Fam Physician 2006; 73 : 1411-6.
12. Vielot, N., et al., The Role of Chlamydia trachomatis in High-Risk Human Papillomavirus Persistence Among Female Sex Workers in Nairobi, Kenya. Sex Transm Dis, 2015. 42(6): p. 305-11.
13. World Health Organization. Global incidence and prevalence of selected curable sexually transmitted infections 2008 [cited 2015 18 Feb].